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Progress Report #2

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EFFECT OF TOBACCO SMOKE AND TOBACCO RESIDUES ON

METHYLCHOLANTHRENE-INDUCED SKIN CARCINOGENESIS IN MICE

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RESULTSTUMOR DEVELOPMENT

At the conclusion of the experiment on November 22nd, 1955, all pertinent data that had been obtained from the weekly record sheets of each animal (refer to accompanying sheet) were assembled and recorded on four large charts (one chart designated for each group), thus presenting in chronological order the progressive changes which developed with respect to the number and size of growths for each animal. From these data, five charts were subsequently constructed and entitled as follows:-

Chart 1 - NUMBER OF TUMORS IN EACH GROUP (Group A)

Chart 2 - NUMBER OF TUMORS IN EACH GROUP (Group B)

Chart 3 - NUMBER OF TUMORS IN EACH GROUP (Group C)

Chart 4 - NUMBER OF TUMORS IN EACH GROUP (Group D)

Chart 5 - NUMBER OF TUMORS IN ALL GROUPS (TOTAL AND AVERAGE)

NOTE:- Only those growths with at least one dimension (width vs. height) of one millimeter or greater were recorded on these charts. Tumors with both dimensions less than one millimeter were extremely difficult to identify correctly.

Graphs, in which the time (in weeks) was plotted against the number of tumors, were reconstructed from these charts and were entitled as follows:-

Graph 1 - AVERAGE NUMBER OF TUMORS PER MOUSE (Group A)

Graph 2 - AVERAGE NUMBER OF TUMORS PER MOUSE (Group B)

Graph 3 - AVERAGE NUMBER OF TUMORS PER MOUSE (Group C)

Graph 4 - AVERAGE NUMBER OF TUMORS PER MOUSE (Group D)

Graph 5 - AVERAGE NUMBER OF TUMORS PER MOUSE (all groups)

Graph 6 - TOTAL NUMBER OF TUMORS (all groups)

Graph 7 - Mortality Rate in All Groups

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group separately in order to demonstrate the sexual variation. The blue line represents the males and the red line the females. The black line represents the total (the sum of the male and female tumors) number of tumors per mouse in that particular group. Graph 5 gives the total number of tumors per mouse (regardless of sex) for all groups and provides a comparison of the groups on the same graph. Graph 6 gives the total number of tumors per group (irrespective of the number of survivors in each group) and shows curves very similar to Graph 5 except in the latter weeks of the experiment. Graph 7 shows the decided increase in mortality during the last few weeks of the experiment. A color key is included in all graphs to identify the various groups.

Number of Tumors in Each Group; Group A, Graph 1:-

Composite results regardless of sex:-

Groups A showed a rapid development in the number of growths commencing on the 10th week and reaching a plateau between the 14th and 15th weeks, and a peak on the 17th week. A sharp decline then occurred to the 18th week, whereupon the number of growths increased again, reaching a second plateau between the 19th and 20th weeks. This was followed by a decline (perhaps due to increased mortality in the latter weeks of the experiment).

Sexual Variations:-

The number of growths induced by the application of methylcholanthrene to the interscapular space of female mice were slightly higher than those recorded for males until the 21st week when the curve for females dropped below the male average.

Number of Tumors in Each Group; Group B, Graph 2:-

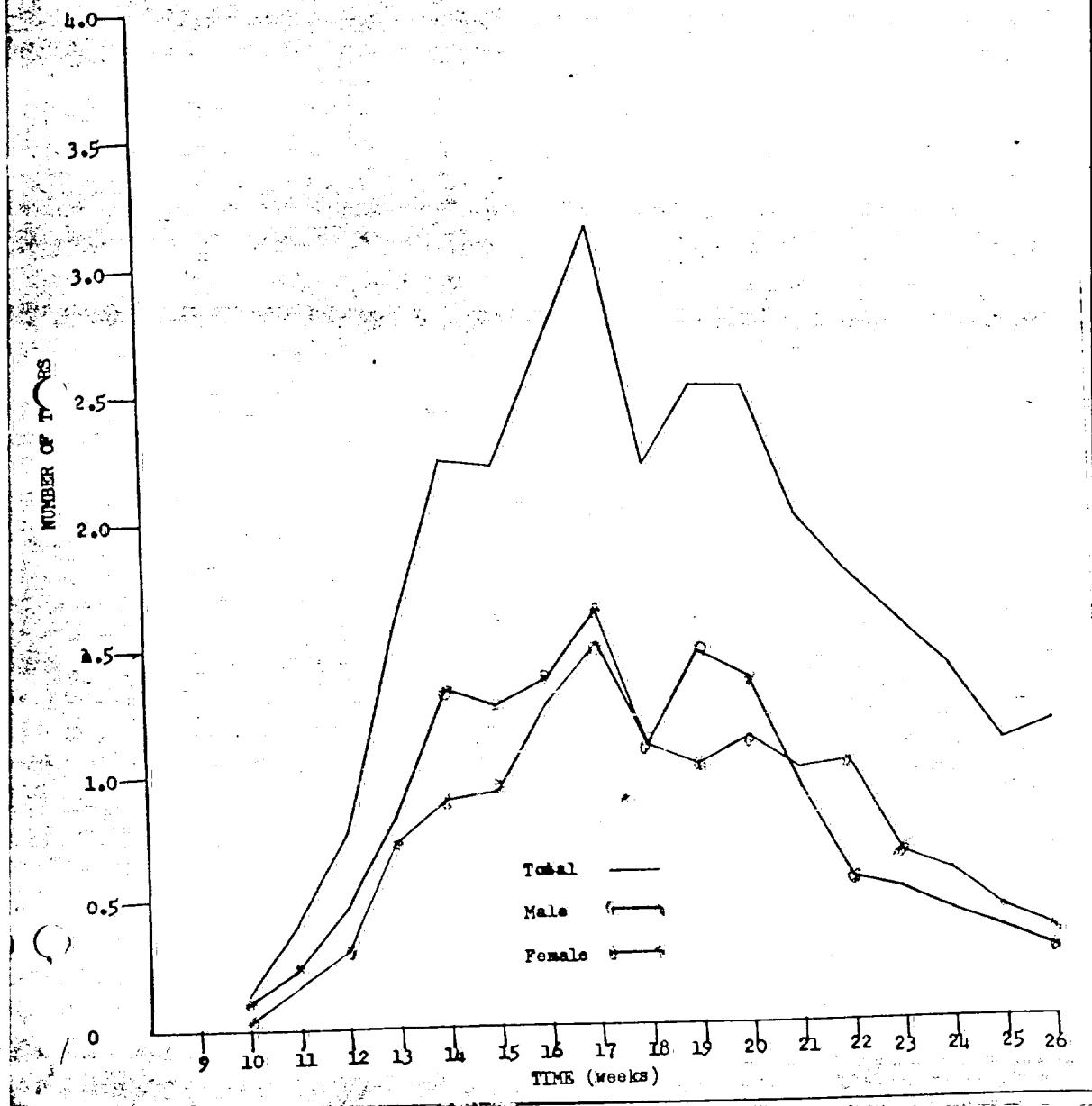
Composite results regardless of sex:-

Group B showed a rapid development starting on the 9th week and reaching the first peak on the 14th week. Then a sharp decline occurred to the 15th week, whereupon the curve rose again, reaching the highest peak on the 17th week. The curve declined gradually to the 22nd week, rising only

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AVERAGE NUMBER OF TUMORS PER MOUSE (Group A)

Graph 1

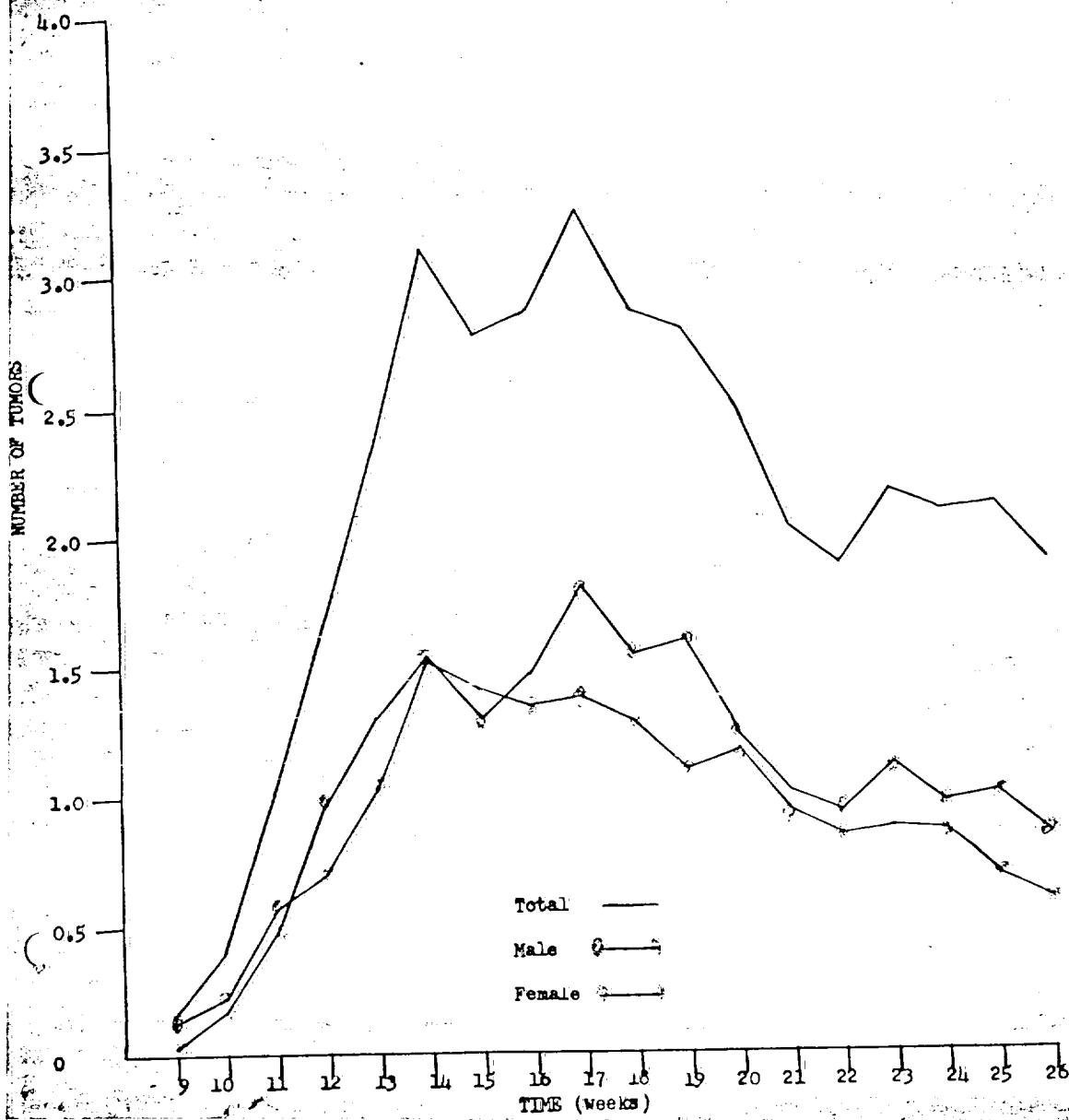


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AVERAGE NUMBER OF TUMORS PER MOUSE (Group B)

Graph 2



slightly to the 23rd week. A plateau then developed to the 25th week, followed by a decline. Group B had a slightly greater number of tumors than A.

Sexual Variations:-

Both sexes showed a similar pattern at the onset. However, after the 15th week, females showed a sharper increase in number of growths than the males.

Number of Tumors in Each Group; Group C, Graph 3:-

Composite results regardless of sex:-

Initial growths occurred during the 9th week of the experiment, whereupon a sharp increase developed reaching the first peak by the 14th week. A decline then occurred to the 15th week followed by a gradual increase to the 18th week. A sharp increase then developed which reached the highest peak on the 20th week. This was followed by a sharp decline to the 22nd week; an increase to the 24th week; leveling off on the 25th week, and finally a rapid decline.

Sexual Variations:-

Both curves were similar until the 13th week. Males then showed a greater number of tumors than females to the 23rd week.

Number of Tumors in Each Group; Group D, Graph 4:-

Composite results regardless of sex:-

Growth began on the 9th week of the experiment. A rapid increase developed shortly after the onset to the 16th week, leveling off to the 19th week. A sharp increase then occurred (slightly higher than for Group C) which attained the highest peak on the 20th week. A decline (resembling that of Group C) then was noted to the 22nd week.

Sexual Variations:-

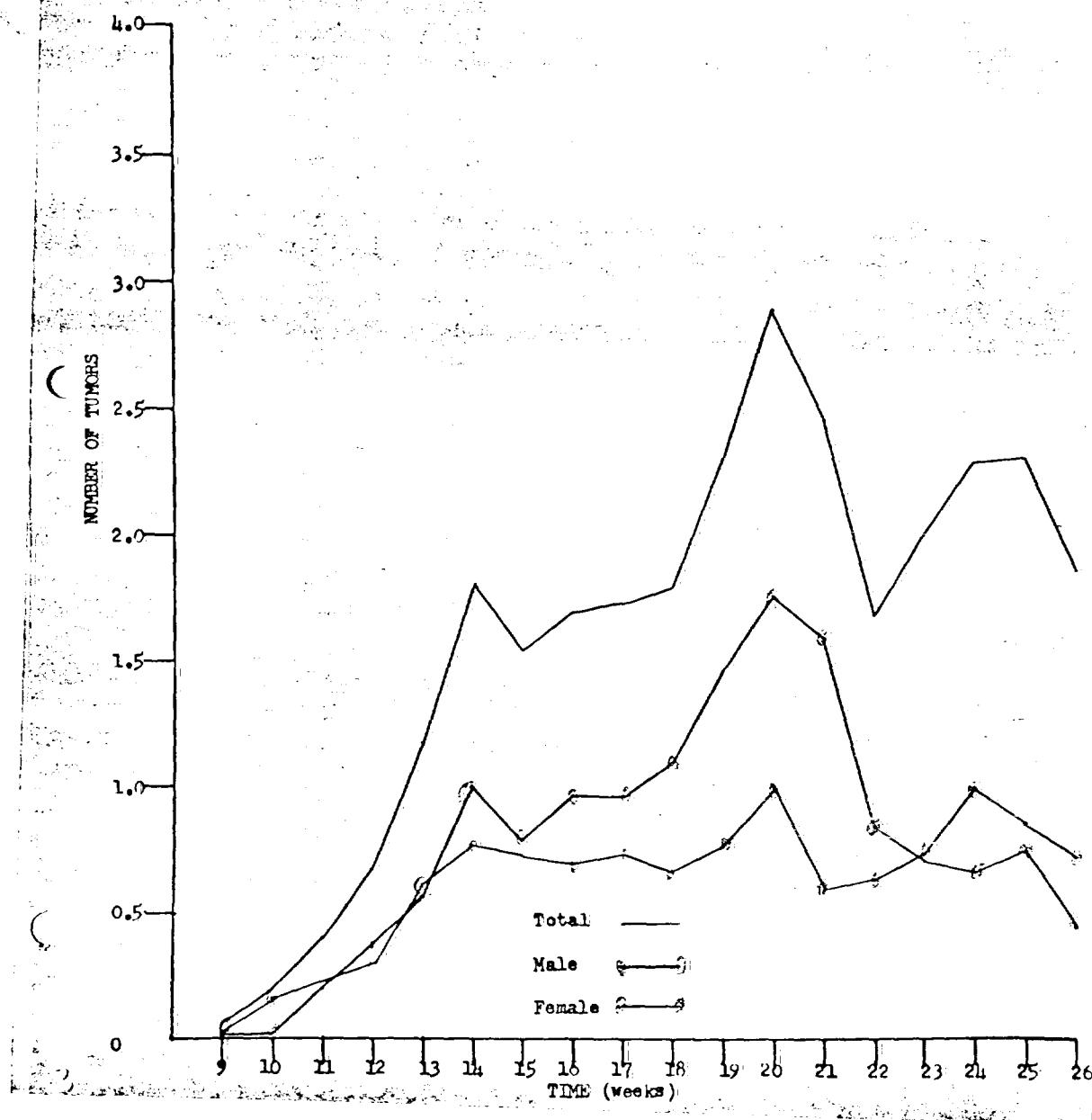
The curves were similar for each sex to the 11th week when males revealed a much greater number of tumors than females to the 25th week.

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AVERAGE NUMBER OF TUMORS PER MOUSE (Group C)

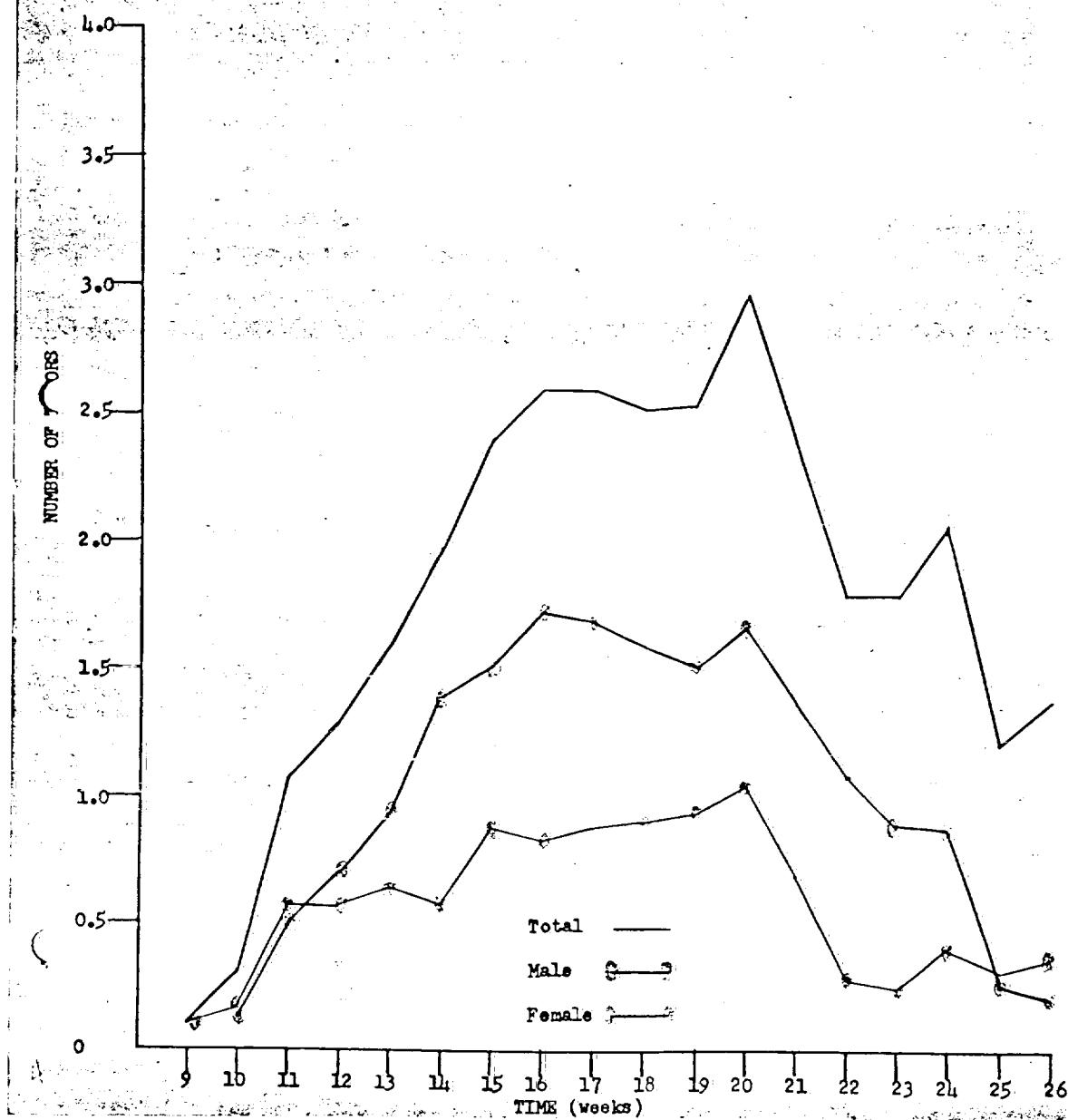
Graph 3



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AVERAGE NUMBER OF TUMORS PER MOUSE. (Group D).

Graph 4



SUMMARY

According to Graph 5, Group C (tobacco residue and methylcholanthrene applications) showed a well-defined inhibition in the development of the number of tumors from the 14th week to the 18th week, and a slower onset of tumor formation than its control group Group D (acetone and methylcholanthrene applications). Group C showed fewer number of tumors than all of the other groups from the 11th week to the 19th week. Groups A, B, and C showed three distinct peaks. These peaks were followed by a decrease or a static condition with respect to the number of growths. This may be due to the superimposition of some defensive response mechanism of the animal to the carcinogen. Group D developed a plateau in place of the initial peak observed in the other groups. During this period the number of growths in the control Group D was appreciably greater than those in Group C. The second peak in Groups A and B occurred three weeks before the second peak of Group C. The third peak of all groups occurred during the latter part of the experiment where the increase in mortality makes the interpretation difficult.

According to the data collected, Group C showed a delay in the onset of tumor formation as compared to its control and had a fewer number of growths than all of the other groups during the period of the experiment when most animals were still alive. It is difficult to draw accurate conclusions from data obtained during the final weeks of the experiment because of the higher mortality rate.

From these data it appears that the biweekly application of tobacco residue definitely inhibits the number of tumors in Group C for a significant period of time, inferring that tobacco residue contains a weak carcinogen with a chemical configuration similar to that of methylcholanthrene.

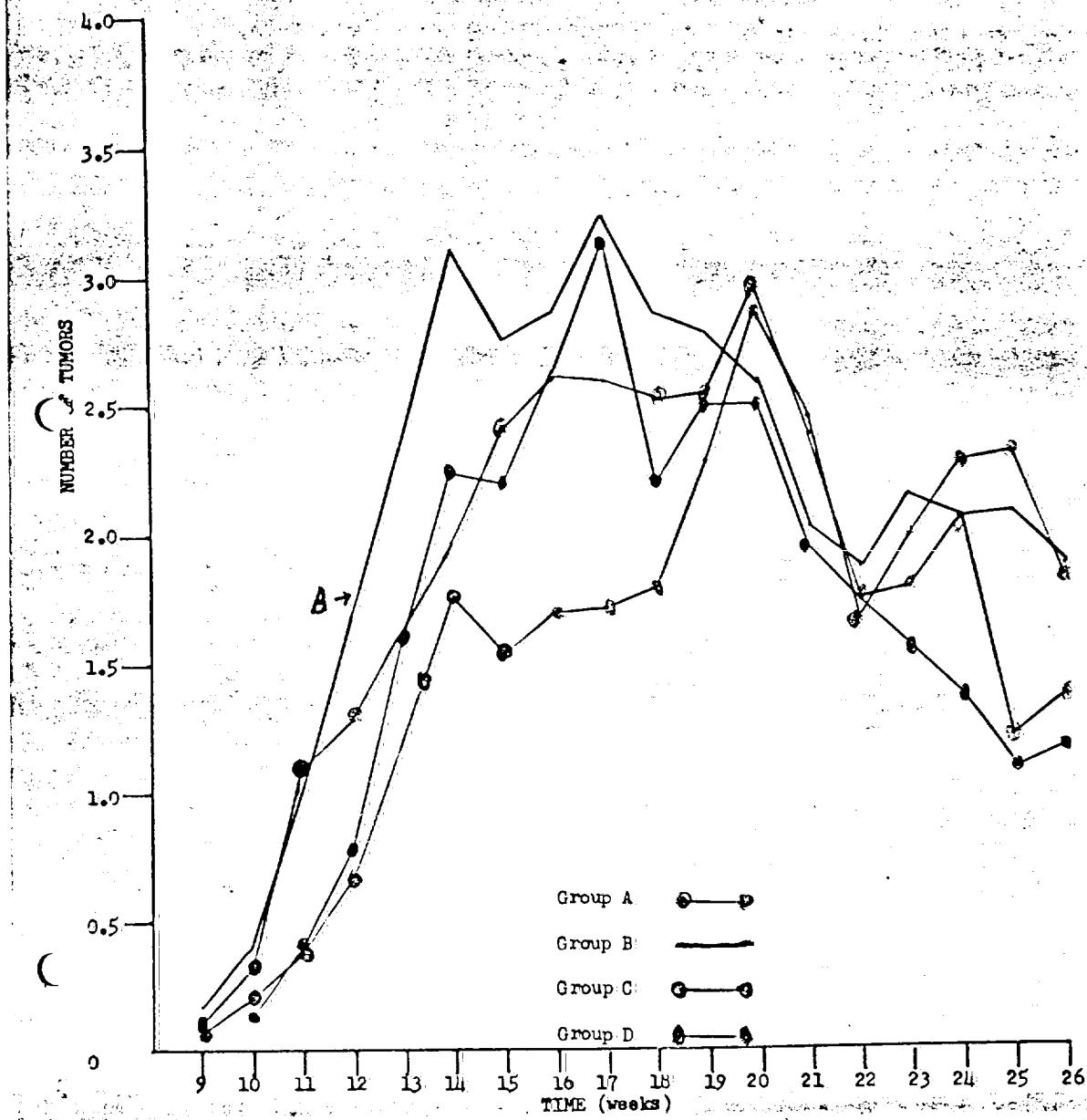
Group B (tobacco smoke and methylcholanthrene) showed the largest number of tumors than any other group until just after the 19th week. It may

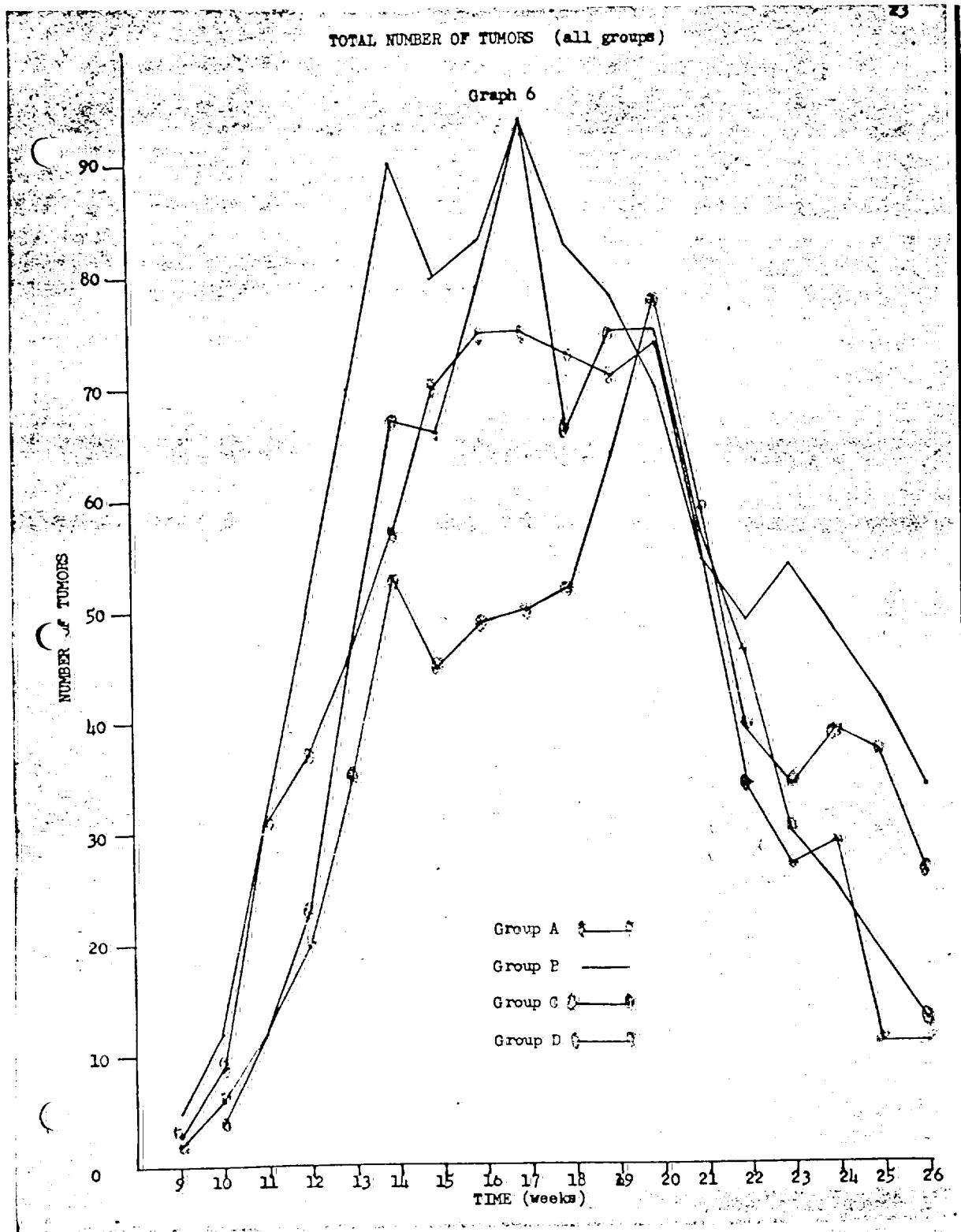
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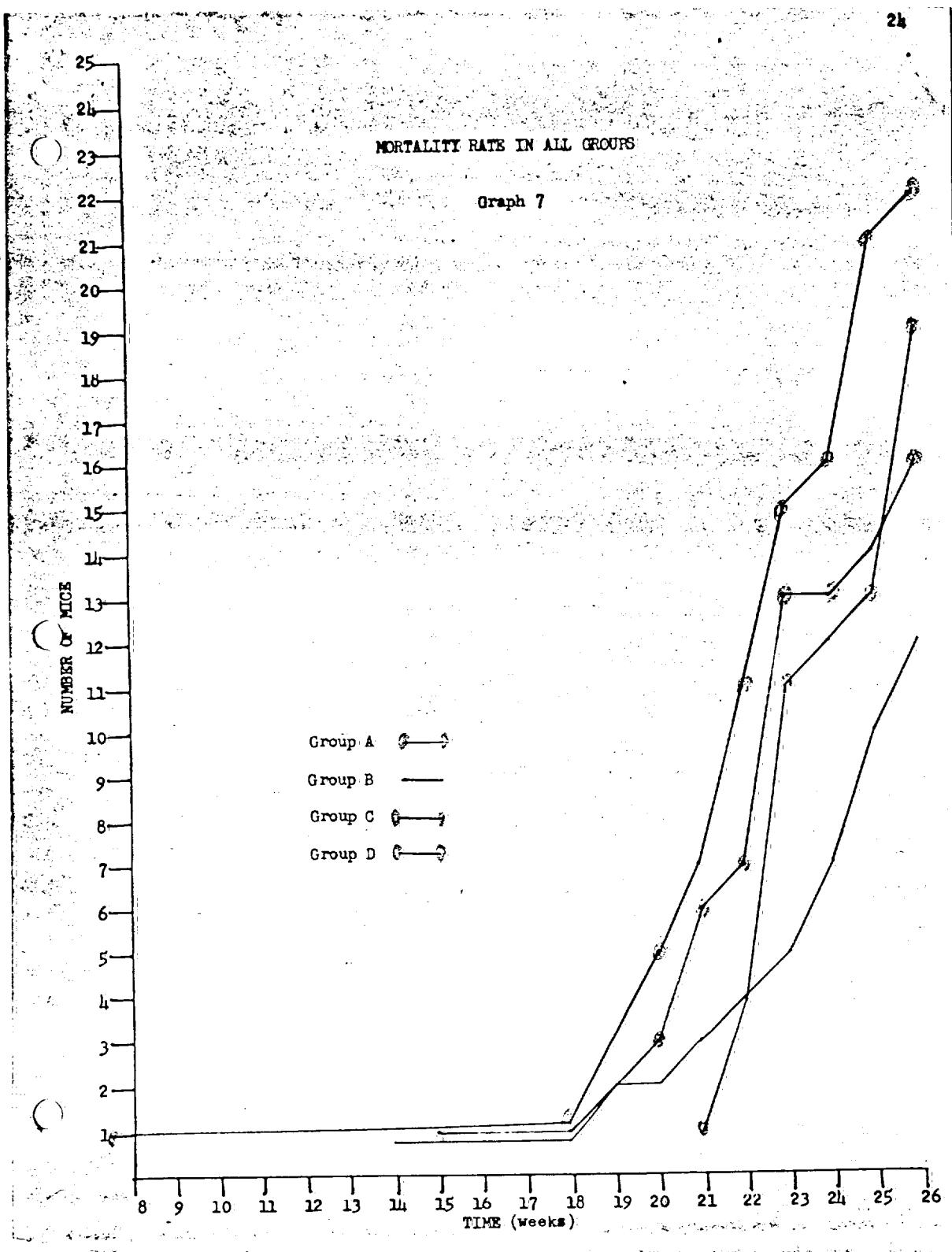
AVERAGE NUMBER OF TUMORS PER MOUSE (all groups)

Graph 5





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be noted that Group B had the lowest mortality after the 22nd week of the experiment.

A more definite conclusion may be drawn after tumor volume has been determined for each animal. This requires a great deal of time as many of the tumors observed were irregular in form. In order to obtain the volume of each tumor of irregular shape it is necessary to reconstruct the growth in clay or similar modeling material from the dimensions and sketches previously taken every week for all animals. This reconstructed tumor in clay or other suitable material can then be weighed and from the density of the material the volume calculated. This necessitates the construction of several hundred models to obtain data of tumor size.

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